which Notch resolves mixed neural identities by repressing an undesired genetic program.

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Program/Abstract # 217
Notch signaling has differing effects on subpopulations of retinal progenitor cells in zebrafish retinal development
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Cell-to-cell interactions mediated by the *Notch* pathway play critical roles in regulating the temporal pattern of neurogenesis. In the vertebrate retina, upon binding a ligand encoded by the *DSL* (*Delta/Serrate/lag-2*) genes, Notch signaling suppresses neuronal differentiation and promotes continued proliferation. Inhibition of Notch signaling results in an increase in the number of neuroblasts that differentiate as ganglion cells and cone photoreceptors, two early cell fates. Conversely, overexpression of a constitutively active form of Notch (NICD) promotes Muller glial differentiation, a later cell fate. Here we tested for a coordinated role of Notch in zebrafish retinal progenitor cell proliferation and subsequent differentiation using the *mindbomb* allele (*mib*^{ta52b}), which lacks Notch function, and two heat-shock transgenic lines that allow for temporal regulation of Notch signaling. In *mib* mutant embryos, BrdU and PH3 labeling revealed that in the absence of Notch signaling, a subset of retinal progenitor cells exits the cell cycle early and differentiates as ganglion cells, while the remainder of progenitor cells continues to proliferate in a spatial and temporal pattern similar to the wild-type pattern. Temporal expression of the NICD resulted in increased Muller glia differentiation at all time points tested as has been previously demonstrated, though mitotic cell numbers were not in excess of their wild-type siblings. Taken together, these data suggest that Notch signaling has differing effects on subpopulations of retinal progenitor cells in zebrafish retinal development.

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Program/Abstract # 218
Lots-of-rods (*lor*) regulates photoreceptor subtype specification in zebrafish
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The number and distribution of neurons generated during development of the vertebrate retina are tightly regulated and critical for image formation. This arrangement is particularly apparent in the highly ordered, crystalline-like mosaic of the photoreceptors in the teleost. Using as a model the mosaic pattern of photoreceptors in the zebrafish, we have undertaken a genetic screen to identify loci that are essential for photoreceptor subtype specification. We identified the locus, *lot-of-rods* (*lor*), that when mutated results in an increased number of rods and a reduced number of ultraviolet-sensitive (UV) cones in larvae and adults. This phenotype is the opposite of that observed in enhanced S-cone syndrome and the rd7 mouse. Quantitative and spatial pattern analyses suggest an approximate one-to-one exchange of rods for UV cones in the mutant compared to wild-type larvae with little alterations in red, green or blue cones. Linkage analysis and complementation testing indicate that the *lor* locus encodes a T-box transcription factor. In genetic chimeras, *lor* mutant cells failed to generate UV cones in a wild-type host. Conversely, wild-type cells displayed the capacity to differentiate into UV cones when transplanted into a mutant host. The identification of a novel function for a T-box gene in photoreceptor development provides a much needed system to understand the molecular network regulating neuronal subtype specification in the retina and dissect the UV vision pathway in a vertebrate. Supported by R1EY017753.

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Program/Abstract # 219
Intra-endodermal interactions are required for pancreatic β-cell induction
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The cellular origin of signals that regulate pancreatic β-cell induction is not clearly defined. Here, we investigate the seeming paradox that Hedgehog/Smoothened signaling functions during gastrulation to promote pancreatic β-cell development in zebrafish, yet has an inhibitory role during later stages of pancreas development in amniotes. Our cell transplantation experiments reveal that in zebrafish, Smoothened function is not required in β-cell precursors. At early somitogenesis stages, when the zebrafish endoderm first forms a sheet, pancreatic β-cell precursors lie closest to the midline; however, the requirement for Smoothened lies in their lateral neighbors, which ultimately give rise to the exocrine pancreas and intestine. Thus, pancreatic β-cell induction requires Smoothened function cell non-autonomously during gastrulation, to allow subsequent intra-endodermal interactions. These results clarify the function of Hedgehog signaling in pancreas development, identify an unexpected cellular source of factors that regulate β-cell specification, and uncover complex patterning and signaling interactions within the endoderm.

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Program/Abstract # 220
PAR-1 phosphorylates the ubiquitin ligase Mind bomb to repress Notch signaling and promote vertebrate neurogenesis
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Epithelial cell polarity is dynamically controlled during early development and is often misregulated in cancer. The serine/threonine kinases PAR-1 and atypical protein kinase C (aPKC) are important players in the establishment of epithelial polarity. Our previous study demonstrated that PAR-1 functions downstream of aPKC to stimulate ciliated cell differentiation in *Xenopus* ectoderm via a Notch signaling-dependent mechanism. Here we show that the same signaling cassette is used during neuronal differentiation of mammalian neural progenitors in vitro. We demonstrate that a crucial molecular substrate for PAR-1 is Mind bomb (MIB), a ubiquitin ligase that promotes Notch signaling by modulating Delta ligand trafficking and activity. The phosphorylation of MIB by PAR-1 results in MIB degradation, repression of Delta-Notch signaling and stimulation of neuronal differentiation. Our data suggest that PAR-1 acts in ectodermal cell fate determination by modulating Notch signaling

via MIB destabilization. We propose that the observed mechanism of binary cell fate determination may have a general significance for asymmetric divisions of polarized progenitor cells in different tissues.

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Program/Abstract # 221
Characterization of calcium channel subunit expression in the developing *Xenopus laevis* nervous system
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A number of studies have linked the specification of neurotransmitter fate to calcium activity in the developing vertebrate nervous system, with the frequency of calcium spikes correlated with specific neurotransmitter fates. However, the specific calcium channel subunits responsible for these transients have yet to be established. Identification and characterization of these calcium channels and their expression patterns will help elucidate the mechanisms by which calcium influences neurotransmitter phenotype development. Using RT-PCR, we have identified calcium channel genes expressed during critical stages of neural development. *In situ* hybridization on late neurula through swimming tadpole embryos has revealed the following expression patterns. Gamma 8 is expressed in the brain, spinal cord, and optic vesicle while alpha 2 delta 3 is expressed in the brain, spinal cord, optic vesicle, and cranial nerve. Alpha 1B is expressed in the midbrain, hindbrain, and cranial nerve, while alpha 1E is expressed in the forebrain. Beta 1 and beta 4 are expressed in the forebrain, midbrain, and bilaterally in the hindbrain. Beta 3 is expressed in the retina and cranial nerve. These data suggest that these subunits may be involved in regulating calcium activity in developing neurons. Fluorescent *in situ* hybridization will allow visualization of coexpression of these subunits with neurotransmitter phenotype markers and will potentially allow us to correlate specific calcium channels with specific neurotransmitter fates.

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Program/Abstract # 222
A novel in vitro system of primary *Xenopus* ectodermal explants to determine the specific function of pan neural SoxB1 proteins
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Currently, the only way to study gene function by overexpression in amphibians is to inject mRNA into whole embryos and assay the effects in the embryo or tissue explants. While this system is very useful, it has some limitations. Injected mRNA is translated immediately and is degraded as the embryo develops. Explants have a finite life span. A result of these limitations is that similar mammalian experiments often do not reconcile with the amphibian data. Our goal is to develop a multipotent *Xenopus* primary culture system that allows us to generate stable cell lines that are hearty, easy to re-establish and can be used to study early cell fate choices. In cell cultures, mRNA can be constitutively expressed via transfected DNA vectors. For example, overexpression of Sox1 or Sox2 in mouse embryonic cell cultures induces neural differentiation. In *Xenopus* explants however, only overexpression of Sox1 mRNA can convert naive ectoderm into neural tissue, whereas Sox2 and Sox3 cannot. An amphibian in vitro model system will bridge the gap between the limitations of amphibian research and the more powerful mammalian in vitro tools. We have shown that *Xenopus* multipotent blastula cells cocultured with an irradiated *Xenopus* fibroblast feeder layer can survive and proliferate in culture. These cells can be transfected with each of the SoxB1 genes and the green fluorescent protein gene. Long-term culture of the embryonic cells under the instructive signals of each of the Sox proteins should recapitulate their specific and potentially unique functions in vivo.

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Program/Abstract # 223
Characterization of neurotransmitter phenotypes in the developing *Xenopus* retina
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The determination of appropriate neurotransmitter phenotypes is a critical aspect of neural development. Because of its organization and accessibility, the developing retina is an excellent system for studying neurotransmitter phenotype determination. To investigate this question in the developing *Xenopus* retina, we are characterizing the expression patterns of a variety of neurotransmitter phenotype markers in the retina during early development. Specifically, we are using multiplex fluorescent *in situ* hybridization to determine the temporal and spatial expression patterns of marker genes for glutamatergic (*xVGlut1*), GABAergic (*xGAT1* and *xGAD67*), and glycinergic (*xGlyT2*) phenotypes in late tailbud to swimming tadpole stage embryos. Confocal analysis of cryosections has allowed us to characterize the expression of each neurotransmitter marker on the cellular level and create a spatial and temporal "map" of neurotransmitter phenotype expression and the co-localization of markers.

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Program/Abstract # 224
Otic placode specification in *Xenopus* by hindbrain-derived signals
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The otic placode is a thickening of the head ectoderm that will develop into the inner ear. Otic placode induction is believed to depend on signals derived from surrounding tissues, the mesoderm and the prospective hindbrain. To define the tissues and signaling molecules involved in this inductive process in *Xenopus*, we tested the ability of segments of the neural plate (NP), isolated from different axial levels, to induce the otic marker Pax8 when recombined with blastula stage animal caps (AC). We found that one single domain of the NP had Pax8-inducing activity in this assay. This NP domain corresponds to a region of the hindbrain expressing high levels of Krox20. Interestingly, a large portion of these recombinants formed otic vesicle-like structures after 24 h in culture. Lineage tracing experiments indicate that these vesicle-like structures are entirely derived from the AC and express several pan-otic markers. Pax8 activation in these recombinants depends on active Fgf and Wnt signaling, as interference with these signaling pathways in the AC blocks Pax8 induction. Wnt1, Wnt8, Fgf3, and Fgf8 are co-expressed in this region of the NP and are therefore good candidates as otic placode inducers. While individual knockdown of these ligands had little effects in the whole embryo, the simultaneous